METHODS AND NUCLEIC ACIDS FOR ANALYSES OF COLORECTAL CELL PROLIFERATIVE DISORDERS

FIELD OF THE INVENTION

The present invention relates to genomic DNA sequences that exhibit altered CpG methylation patterns in disease states relative to normal. Particular embodiments provide methods, nucleic acids, nucleic acid arrays and kits useful for detecting, or for detecting and differentiating between or among colorectal cell proliferative disorders.

SEQUENCE LISTING

A Sequence Listing, pursuant to 37 C.F.R. § 1.52(e)(5), has been provided on compact disc (1 of 1) as a 3.048 MB file, entitled 47675-47.txt, and which is incorporated by reference herein in its entirety.

BACKGROUND

The etiology of pathogenic states is known to involve modified methylation patterns of individual genes or of the genome. 5-methylcytosine, in the context of CpG dinucleotide sequences, is the most frequent covalently modified base in the DNA of eukaryotic cells, and plays a role in the regulation of transcription, genetic imprinting, and tumorigenesis. The identification and quantification of 5-methylcytosine sites in a specific specimen, or between or among a plurality of specimens, is thus of considerable interest, not only in research, but particularly for the molecular diagnoses of various diseases.

Correlation of aberrant DNA methylation with cancer. Aberrant DNA methylation within CpG 'islands' is characterized by hyper- or hypomethylation of CpG dinucleotide sequences leading to abrogation or overexpression of a broad spectrum of genes, and is among the earliest and most common alterations found in, and correlated with human malignancies. Additionally, abnormal methylation has been shown to occur in CpG-rich regulatory elements in intronic and coding parts of genes for certain tumors. In colon cancer, for example, aberrant DNA methylation constitutes one of the most prominent alterations and inactivates

many tumor suppressor genes such as p14ARF, p16INK4a, THBS1, MINT2, and MINT31 and DNA mismatch repair genes such as hMLH1.

In contrast to the specific hypermethylation of tumor suppressor genes, an overall hypomethylation of DNA can be observed in tumor cells. This decrease in global methylation can be detected early, far before the development of frank tumor formation. A correlation between hypomethylation and increased gene expression has been determined for many oncogenes.

Colorectal Cancer. Colorectal cancer is the fourth leading cause of cancer mortality in men and women, although ranking third in frequency in men and second in women. The 5-year survival rate is 61% over all stages with early detection being a prerequisite for curative therapy of the disease. Up to 95% of all colorectal cancers are adenocarcinomas of varying differentiation grades.

Sporadic colon cancer develops in a multistep process starting with the pathologic transformation of normal colonic epithelium to an adenoma which consecutively progresses to invasive cancer. The progression rate of benign colonic adenomas depends strongly on their histologic appearance: whereas tubular-type adenomas tend to progress to malignant tumors very rarely, villous adenomas, particularly if larger than 2 cm in diameter, have a significant malignant potential.

During progression from benign proliferative lesions to malignant neoplasms several genetic and epigenetic alterations occur. Somatic mutation of the APC gene seems to be one of the earliest events in 75 to 80% of colorectal adenomas and carcinomas. Activation of K-RAS is thought to be a critical step in the progression towards a malignant phenotype. Consecutively, mutations in other oncogenes as well as alterations leading to inactivation of tumor suppressor genes accumulate.

In the molecular evolution of colorectal cancer, DNA methylation errors have been suggested to play two distinct roles. In normal colonic mucosa cells, methylation errors accumulate as a function of age or as time-dependent events predisposing these cells to neoplastic transformation. For example, hypermethylation of several loci could be shown to be already present in adenomas, particularly in the tubulovillous and villous subtype. At later

stages, increased DNA methylation of CpG islands plays an important role in a subset of tumors affected by the so called CpG island methylator phenotype (CIMP). Most CIMP+ tumors, which constitute about 15% of all sporadic colorectal cancers, are characterized by microsatellite instability (MIN) due to hypermethylation of the hMLH1 promoter and other DNA mismatch repair genes. By contrast, CIMP- colon cancers evolve along a more classic genetic instability pathway (CIN), with a high rate of p53 mutations and chromosomal changes.

However, the molecular subtypes do not only show varying frequencies regarding molecular alterations. According to the presence of either micro satellite instability or chromosomal aberrations, colon cancer can be subclassified into two classes, which also exhibit significant clinical differences. Almost all MIN tumors originate in the proximal colon (ascending and transversum), whereas 70% of CIN tumors are located in the distal colon and rectum. This has been attributed to the varying prevalence of different carcinogens in different sections of the colon. Methylating carcinogens, which constitute the prevailing carcinogen in the proximal colon have been suggested to play a role in the pathogenesis of MIN cancers, whereas CIN tumors are thought to be more frequently caused by adduct-forming carcinogens, which occur more frequently in distal parts of the colon and rectum. Moreover, MIN tumors have a better prognosis than do tumors with a CIN phenotype and respond better to adjuvant chemotherapy.

Incidence and mortality rates for this disease increase greatly with age, particularly after the age of 60. Stage of disease at diagnosis also affects overall survival rates. Patients having lesions confined to the colonic wall have a high probability of surviving 5 or more years while patients with metastatic disease have a very low probability of survival. It is thought that most colorectal cancers develop over a course of 5-10 years from a precursor lesion called an adenomatous polyp. The potential of these lesions to result in adenocarcinoma has been shown to increase with both polyp size and degree of dysplasia. Because of the slow progression of this disease, early detection through routine screening can result in significant improvement of survival rates. Several randomized trials over the last 20 years have shown that screening test can reduce mortality over 30%, even though the tests

used were not highly sensitive. The current guidelines for colorectal screening according to the American Cancer Society utilizes one of five different options for screening in average risk individuals 50 years of age or older. These options include 1) fecal occult blood test (FOBT) annually, 2) flexible sigmoidoscopy every five years, 3) annual FPBT plus flexible sigmoidoscopy every five years, 4) double contrast barium enema (DCBE) every five years or 5) colonoscopy every ten years. Even though these testing procedures are well accepted by the medical community, the implementation of widespread screening for colorectal cancer has not been realized. Patient compliance is a major factor for limited use due to the discomfort or inconvenience associated with the procedures. FOBT testing, although a non-invasive procedure, requires dietary and other restrictions 3-5 days prior to testing. Sensitivity levels for this test are also very low for colorectal adenocarcinoma with wide variability depending on the trial. Sensitivity measurements for detection of adenomas is even less since most adenomas do not bleed. In contrast, sensitivity for more invasive procedures such as sigmoidoscopy and colonoscopy are quite high because of direct visualization of the lumen of the colon. No randomized trials have evaluated the efficacy of these techniques, however, using data from case-control studies and data from the National Polyp Study (U.S.) it has been shown that removal of adenomatous polyps results in a 76-90% reduction in CRC incidence. Sigmoidoscopy has the limitation of only visualizing the left side of the colon leaving lesions in the right colon undetected. Both scoping procedures are expensive, require cathartic preparation and have increased risk of morbidity and mortality. Improved tests with increased sensitivity, specificity, case of use and decreased costs are clearly needed before general widespread screening for colorectal cancer becomes routine.

Molecular disease markers offer several advantages over other types of markers, one advantage being that even samples of very small sizes and/or samples whose tissue architecture has not been maintained can be analyzed quite efficiently. Within the last decade a number of genes have been shown to be differentially expressed between normal and colon carcinomas. However, no single or combination of marker has been shown to be sufficient for the diagnosis of colon carcinomas. High-dimensional mRNA based approaches have recently been shown to be able to provide a better means to distinguish between different tumor types